

# Validation and standardization of Kveim test suspensions prepared from two human sarcoid spleens

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**SYNOPSIS** Single lots of a Chase-Siltzbach type I Kveim test material from each of two sarcoid spleens and designated lot 5 of spleen K12 and lot 1 of spleen K13 have been validated alongside a single lot (lot 10) of a 'standard' suspension provided by Dr L. E. Siltzbach and prepared identically from the spleen of patient J (spleen J) in New York. Additionally, a half-dilution of lot 5, K12, was included in this comparison. The reactivity of each suspension was assessed among patients with active and inactive sarcoidosis. The selectivity of each suspension for sarcoidosis was assessed similarly by comparison with results in patients with active and quiescent pulmonary parenchymal tuberculosis and in healthy subjects. All patients were closely matched and two Kveim tests were made in each subject according to a prearranged statistical design. The reactivity of the K12, K12½ dilution, and K13 suspensions among patients with active and inactive sarcoidosis was closely similar to that with the 'standard' S10 suspension and in accordance with the expected proportions of reactions in patients at different stages of sarcoidosis. The K12, K13, and 'standard' S10 suspensions yielded a negligible proportion of positive reactions among patients with active and quiescent pulmonary tuberculosis and among healthy subjects: thus, as judged by these tests each suspension showed a high degree of selectivity for sarcoidosis.

The results of this validation study are discussed in relation to the results of other studies in which lots 5 and 14 of K12 and early and late batches of a suspension prepared from another sarcoid spleen at the Commonwealth Serum Laboratories designated CSL and provided by Dr T. H. Hurley in Melbourne were employed. Using lot 5 of K12 positive reactions were found in an appreciable proportion of patients with Crohn's disease, ulcerative colitis, and tuberculous lymphadenitis. A closely similar rate of positive reactions was encountered among patients with Crohn's disease following tests with batch 0025 of CSL suspension and with another lot (lot 14) derived from spleen K12. A close concordance of results was obtained with lot 5 (K12) and with batch 0042 CSL among patients with ulcerative colitis, but at a lower rate of reactivity.

We conclude that positive reactions also occur in some diseases other than sarcoidosis and consider that the difficulties in determining the criteria for an acceptable test suspension become increasingly apparent as additional Kveim tests are made with one particular lot and with sequential lots of material from a 'validated' tissue source.

The human sarcoid spleen represents the best source of Kveim test material. Although satisfactory test suspensions can also be prepared from other tissues affected by sarcoidosis, a spleen, once it has been validated, will by virtue of its size ensure an ample

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supply of sequential lots of potentially acceptable test materials for general use over a prolonged period. Unfortunately, the histological appearances of a sarcoid spleen give no guidance to either the reactivity or the selectivity of suspensions prepared from it; these can be determined only by biological assay

in man. Kveim tests in patients with definite clinical sarcoidosis will determine the *reactivity* of the suspension: comparison with identical tests among patients with other granulomatous and non-granulomatous diseases (particularly tuberculosis) and among healthy subjects will determine its *selectivity* for sarcoidosis.

### Objectives

The objectives of the present study were to assess the macroscopic and microscopic response at the Kveim test site four to six weeks after the intracutaneous injection of Kveim test suspensions prepared from two human sarcoid spleens, in comparison with the reactions to a standard validated suspension. Tests were made in patients with active sarcoidosis, inactive sarcoidosis, active and quiescent pulmonary parenchymal tuberculosis, and in healthy subjects.

### MATCHING OF SUBJECTS

Each patient with clinically active sarcoidosis was given two simultaneous Kveim tests according to a prearranged statistical design, and the same pair of simultaneous tests was given at the same sites in one subject in each of the following categories:

- 1 a patient with definite but inactive sarcoidosis matched for:
  - (a) sex,
  - (b) age on initial diagnosis (to within 5 years),
  - (c) form of disease present at the time of the first abnormal chest radiograph,
  - (d) tuberculin status at the time of the first abnormal chest radiograph (when possible);
- 2 a patient with confirmed active pulmonary tuberculosis matched for:
  - (a) sex,
  - (b) age on initial diagnosis (to within 5 years);
- 3 a patient with confirmed quiescent pulmonary parenchymal tuberculosis matched for:
  - (a) sex,
  - (b) age on initial diagnosis (to within 5 years);
- 4 an apparently healthy subject matched for:
  - (a) sex,
  - (b) age of the patient with active sarcoidosis on initial diagnosis (to within 5 years).

### Material and Methods

#### TEST SPLEENS

##### *Spleen K12*

A spleen was obtained at splenectomy for hypersplenism from a woman aged 35 years with hilar lymphadenopathy and widespread pulmonary infiltration attributed to sarcoidosis. A scalene node

biopsy had shown non-caseating giant-cell granulomas and microscopically the Kveim test was weakly positive.

##### *Spleen K13*

A sarcoid spleen was obtained at necropsy from a woman aged 74 years whose chest radiograph showed hilar lymphadenopathy and pulmonary infiltration with fibrosis attributed to sarcoidosis. A scalene node biopsy had shown non-caseating giant-cell granulomas but the Kveim test was microscopically negative.

#### STANDARD SUSPENSION

This suspension (designated S10) was supplied by Dr L. E. Siltzbach from lot 10 of a validated spleen (spleen J). It contained 450  $\mu$ g alcohol-precipitable material per test dose.

#### TEST SUSPENSIONS

The three test suspensions were all of type 1 (Chase-Siltzbach) prepared according to the technique of Chase (1961) in a manner identical with that of S10. They were:

K12 undiluted—This lot (lot 5) contained 1250  $\mu$ g per test dose.

K12  $\frac{1}{2}$  dilution—This was a half dilution of K12 (lot 5).

K13 undiluted—This lot (lot 1) contained 880  $\mu$ g per test dose.

Each suspension was dispensed in identical rubber-capped glass vials (2 ml) identified only by a code number to conceal the nature of the suspension as far as possible from the tester and the readers.

#### THE KVEIM TEST

The dose of each Kveim reagent was that contained in 0.15 ml of the test suspension, drawn into a 1 ml tuberculin-type disposable syringe after shaking the vial to ensure a uniform suspension. The injections were made through a 26 gauge needle, through which each particulate suspension was found to pass freely.

##### *The Kveim test sites*

The two tests on each patient were made intracutaneously on the ulnar aspect of the forearm. The two sites were chosen at random from four possible standard test sites, two on each forearm. All the tests were made by one person (DNM).

##### *Method of locating test sites*

The location of each Kveim test was identified by a single tattoo speck of autoclaved Gunther-Wagner Pelikan Ink just breaking the epidermis within the medial edge of each injected weal.

### *Reading of the Kveim test sites*

Each test site was examined carefully immediately before biopsy, that is, at four to six weeks following injection. The maximum diameter of any papule at each test site was measured with calipers and recorded in millimetres (by DNM).

### *Biopsy of Kveim test site*

A full thickness skin biopsy at each of the two test sites was made in each patient four to six weeks after injection. All the biopsies were made by one person (DNM). The skin at each test site was cleaned with 70% alcohol, and 2% xylocaine with adrenaline was then injected into the skin at the test site. A Hayes Martin drill biopsy punch with a 4 mm knife was used to excise the full thickness of skin at the injection site, the knife removing a core of skin extending down to the subcutaneous tissue. A 'butterfly' band-aid was then used to appose the edges of the wound and avoid the need for sutures. Each biopsy specimen was transferred to 10% formalin and prepared histologically. Serial sections were prepared, and every twentieth was mounted and stained with haematoxylin and eosin.

### *Microscopic assessment of Kveim test biopsies*

The sections from each Kveim test biopsy were read independently, according to the criteria in the Appendix, by two observers (JGS and DNM), who were unaware of the nature of the suspension or the clinical status of the subjects.

### **TUBERCULIN TESTS**

To assist the matching process, a tuberculin test was made where possible with 0.1 ml of 1/3000 Old Tuberculin (3 TU) in all patients with clinically active or inactive sarcoidosis. The test site was distinct from those for the Kveim tests. The tuberculin test was given at the same time as the two Kveim tests and was read at three days. The maximum diameter of induration was recorded in millimetres. A tuberculin test with 100 TU was made at a site distinct from those of previous tests in all patients in whom the reaction to 3 TU was less than 5 mm. These tests were also read at three days, and the maximum diameter of any induration was recorded in millimetres.

### **DESIGN OF STUDY**

The study was made on 18 sets of five subjects, each set consisting of one patient with active sarcoidosis, one with inactive sarcoidosis, one with active and one with quiescent pulmonary tuberculosis, and one healthy subject, matched according to the criteria stated above. There are six different combinations of the four tests taken in pairs, and each combination

was tested on three patients with active sarcoidosis and on the subjects matched with them. Thus each of the four suspensions was tested on nine patients with active sarcoidosis (and their matches).

### **Results**

#### **MICROSCOPIC READINGS OF KVEIM TEST RESULTS**

Because of the involvement of the other reader (DNM) in the test and biopsy procedures, the microscopic results presented here are based on the readings of one reader only (JGS). The two sets of readings were, however, closely similar. There was complete agreement of reading in 87% of the total of 180 biopsies, and in only four of the 24 disagreements was there a major difference (that is, one reading 'positive', the other 'negative'), the remainder all involving the 'equivocal' category.

#### *Reactivity (table I)*

Among the 18 patients with active sarcoidosis 12 (67%) had at least one positive biopsy (the companion biopsy was positive in 7, equivocal in 2, and negative in 3). Among the 18 patients with inactive sarcoidosis four (22%) had at least one positive biopsy (the companion biopsy was positive in 1 and equivocal in 3); in addition, one patient had an equivocal reading for both biopsies. A comparison of the reactions in the matched pairs of patients with active and inactive sarcoidosis, scoring  $\frac{1}{2}$  for each positive and  $\frac{1}{4}$  for each equivocal reading, shows a significantly greater reactivity of the Kveim test in the patients with active sarcoidosis than in those with inactive sarcoidosis ( $P < 0.05$ , Wilcoxon rank test).

#### *Selectivity (table I)*

Among the 36 patients with pulmonary tuberculosis (18 active, 18 quiescent) none had a positive biopsy. Among the 18 healthy subjects one had a positive biopsy (the companion being negative).

#### *Reactivity of the four test suspensions*

Table II summarizes the readings in each group of patients according to the test suspension. There are no obvious differences in reactivity between the S10, K12, and K12  $\frac{1}{2}$  suspensions in either active or inactive sarcoidosis, but the figures in the table suggest that suspension K13 may have been rather less reactive than the other three. However, a detailed analysis of the differences between the reactions to the pair of suspensions used on each patient does not show any significant differences between the four suspensions.

Subjects	Total Patients	Readings of the Two Biopsies						Patients with a Positive Result (%)
		Positive Positive	Positive Equivocal	Positive Negative	Equivocal Equivocal	Equivocal Negative	Negative Negative	
Active sarcoidosis	18	7	2	3	0	0	6	67
Inactive sarcoidosis	18	1	3	0	1	1	12	22
Active pulmonary tuberculosis	18	0	0	0	0	1	17	0
Quiescent pulmonary tuberculosis	18	0	0	0	0	2	16	0
Healthy	18	0	0	1	0	1	16	6

Table I Results of microscopic readings of the two Kveim test biopsies in each patient

Subjects	Suspension	Biopsies			
		Total	Positive	Equivocal	Negative
Active sarcoidosis	S10	9	5	1	3
	K12	9	6	0	3
	K12½	9	5	0	4
	K13	9	3	1	5
	Total	36	19	2	15
Inactive sarcoidosis	S10	9	2	4	3
	K12	9	1	2	6
	K12½	9	2	0	7
	K13	9	0	0	9
	Total	36	5	6	25
Pulmonary tuberculosis (active and quiescent)	S10	18	0	2 <sup>1</sup>	16
	K12	18	0	1	17
	K12½	18	0	0	18
	K13	18	0	0	18
	Total	72	0	3	69
Healthy	S10	9	0	0	9
	K12	9	1	0	8
	K12½	9	0	0	9
	K13	9	0	1	8
	Total	36	1	1	34

Table II Results of microscopic readings of each Kveim test biopsy according to test suspension

<sup>1</sup>Including the one equivocal result in a patient with active pulmonary tuberculosis

#### READINGS OF PAPULE SIZE

Table III summarizes the diameters of the Kveim test papules in each group of subjects according to the test suspension. The mean papule sizes were significantly larger in patients with active sarcoidosis than in those with inactive sarcoidosis (3.4 mm compared with 1.8 mm,  $P < 0.001$ ). In all, papules were found in 41 of the 72 test sites in patients with pulmonary tuberculosis, and in only one of the 36 test sites in healthy subjects.

There are no obvious differences in mean papule size between the four suspensions within each group of patients.

#### PAPULE SIZE IN RELATION TO THE MICROSCOPIC READING

Table IV shows that there is a close correlation between the papule size and the microscopic reading ( $P < 0.001$ ). The mean papule diameters were 3.8, 2.4, and 1.1 mm for those positive, equivocal, and negative on biopsy, respectively.

It is of interest that the mean papule sizes for the microscopically *negative* biopsies (as for all biopsies) were greater in patients with active sarcoidosis than in those with inactive sarcoidosis or pulmonary tuberculosis, and that these were in turn greater than in healthy subjects (table V). This indicates that the macroscopic as well as the microscopic reading is relevant to a full interpretation of the result of the Kveim test.

#### TUBERCULIN TESTS IN THE SARCOIDOSIS PATIENTS

Of the 18 patients with active sarcoidosis (table VI), six were positive to 3 TU, five were positive to 100 TU only, and seven were negative to 3 TU and 100 TU. Of the 18 patients whose sarcoidosis was inactive at the time of testing, four were positive to 3 TU, three were positive to 100 TU only, and 11 were negative to 3 and 100 TU. Tuberculin tests were not made in connection with this study in the patients with tuberculosis or in the healthy subjects.

Subjects	Suspension	Total Papules	Diameter (mm)							Mean
			6	5	4	3	2	1	0	
Active sarcoidosis	S10	9	0	4	2	2	1	0	0	4.0
	K12	9	0	1	2	3	1	1	1	2.8
	K12½	9	1	1	3	1	3	0	0	3.6
	K13	9	0	1	3	3	2	0	0	3.3
	Total	36	1	7	10	9	7	1	1	3.4
Inactive sarcoidosis	S10	9	0	0	1	4	2	0	2	2.2
	K12	9	0	0	2	2	0	0	5	1.6
	K12½	9	0	0	2	1	1	1	4	1.6
	K13	9	0	0	0	5	1	0	3	1.9
	Total	36	0	0	5	12	4	1	14	1.8
Pulmonary tuberculosis (active and quiescent)	S10	18	0	0	0	2	5	4	7	1.1
	K12	18	0	0	1	2	3	3	9	1.1
	K12½	18	0	0	1	1	2	5	9	0.9
	K13	18	0	0	1	5	6	0	6	1.7
	Total	72	0	0	3	1	16	12	31	1.2
Healthy	S10	9	0	0	0	0	0	0	9	0.0
	K12	9	0	0	0	0	0	0	9	0.0
	K12½	9	0	0	0	0	0	0	9	0.0
	K13	9	0	0	0	1	0	0	8	0.3
	Total	36	0	0	0	1	0	0	35	0.1

Table III Diameter of Kveim test papules according to test suspension

Microscopic Reading	Total Papules	Diameter (mm)							Mean
		6	5	4	3	2	1	0	
Positive	25	1	7	1	5	0	0	2	3.8
Equivocal	12	0	0	3	4	2	0	3	2.4
Negative	143	0	0	5	23	25	14	76	1.1

Table IV Diameter of Kveim test papules according to microscopic readings

Subjects	Microscopic Reading						All Subjects	
	Positive		Equivocal		Negative			
	No. Papules	Mean Diam.	No. Papules	Mean Diam.	No. Papules	Mean Diam.	No. Papules	Mean Diam.
Active sarcoidosis	19	4.3	2	4.0	15	2.2	36	3.4
Inactive sarcoidosis	5	2.8	6	2.5	25	1.5	36	1.8
Pulmonary tuberculosis (active and quiescent)	0	—	3	1.7	69	1.3	72	1.2
Healthy	1	0.0	1	0.0	34	0.1	36	0.1
All	25	3.8	12	2.4	143	1.1	180	1.5

Table V Mean diameter of Kveim test papules according to microscopic readings in each group of patients

Subjects	Total tested	Negative to 3 TU		
		Positive to 100 TU	Negative to 100 TU	Positive to 3 TU
Active sarcoidosis	18	5	7	6
Inactive sarcoidosis	118	3	11	4

Table VI Tuberculin sensitivity in the sarcoidosis patients

## Discussion

The results obtained with the K12 suspension in this study show that this Kveim test material is reactive in sarcoidosis and is also highly selective for this disease. A positive Kveim reaction was

obtained in 6 of 9 patients with active sarcoidosis. In patients with inactive sarcoidosis at the time of testing, 1 of 9 showed a positive Kveim test. The corresponding figures for the half-dilution of K12 were 5 of 9 and 2 of 9. These findings are closely similar to those with the 'standard' S10 suspension

in this study, namely 5 of 9 patients with active, and 2 of 9 patients with inactive sarcoidosis. Hirsch *et al* (1961) reported the results of a study in which a Kveim suspension, again a type I of Chase and Siltzbach similar to the 'standard' S10 suspension used in the present study, was likewise injected intracutaneously into persons with sarcoidosis and various other diseases. Again the test sites were removed by punch biopsy four to six weeks after injection, and sections of these biopsies were assessed by two observers who were unaware of the clinical diagnosis. A positive Kveim test was found in 75% of 71 patients with subacute active sarcoidosis and in 64% of 45 patients in whom active sarcoidosis was known to have been present for more than two years. The lower reactivity in subjects with inactive sarcoidosis appears to be related to the interval since onset of the disease (Siltzbach, 1961a, b).

With regard to the selectivity of the K12 suspension, one positive reaction was found among nine apparently healthy subjects and none among 18 patients with pulmonary tuberculosis. With the half-dilution, the figures were 0 of 9 and 0 of 18. Similarly, Hirsch *et al* (1961) found positive Kveim reactions in less than 5% of 43 subjects with arrested tuberculosis or connective tissue disease, and among young schizophrenic men who had negative tuberculin tests.

The findings for the K12 suspension are similar to the more recent findings of the international Kveim test study reported by Siltzbach (1967) in which, of those with biopsy confirmed sarcoidosis of less than two years' duration, 62% gave a positive reaction following tests with his suspension. Among those with 'chronic' lesions, 38% responded, demonstrating again the waning of Kveim reactivity with the passage of time; only two (1.2%) positive reactions were seen, both in patients with leprosy, of the 173 subjects tested in the control group.

The findings of a negative Kveim test among some patients with recent active sarcoidosis remains unexplained. None of the patients in the present study were receiving corticosteroids during the period of Kveim testing, which is known to depress the Kveim reaction (James and Thompson, 1955; Nelson, 1957; Siltzbach, 1961a, b).

The concentration of the Chase-Siltzbach suspensions used in this study varied widely. Thus, in tests with standard S10 material 450  $\mu\text{g}$  was deposited at each test site whereas in tests with K12 no less than 1250  $\mu\text{g}$  of alcohol-precipitable material was deposited at each test site. For K13 this figure was 880  $\mu\text{g}$ . Despite these wide differences, the levels of reactivity of K12 and S10 were closely similar and that for K13 was only slightly less. Hence the

alcohol-precipitable dry weight of test material can serve only as a very approximate guide to the activity of a test suspension which can be assessed only following biological assay in patients with sarcoidosis. Similarly, it is not possible to assess reliably, before validation in man, which sarcoid spleen will yield a potent test suspension. Thus Siltzbach (1964) reported his evaluation of 38 different tissue suspensions, of which only 18 were satisfactory. The remaining 20 were either too weak or elicited non-specific granulomata. Putkonen (1943; 1964) stressed that the most active Kveim test suspensions were frequently obtained from patients whose Kveim test showed only a weak positive reaction, and it is of interest that the Kveim test in the donor of spleen K12 was only weakly positive and that for K13 was negative.

The suspension prepared from spleen K13 appeared to be slightly less reactive than spleen K12 or S10 as a source of Kveim test material, but the differences are not significant. K13 is a relatively small spleen and is for this reason less suited to yield a continued supply of validated Kveim material.

This study has in general confirmed the reactivity and selectivity of all the Kveim test suspensions in patients with active sarcoidosis but has not indicated whether these suspensions differ in their reactivity. It has also confirmed the greater reactivity of the Kveim test suspensions in active than in inactive sarcoidosis.

The findings on papule size are of interest in two respects. First, papules were found in 41 of 72 Kveim tests in patients with pulmonary tuberculosis, although none of these tests was positive on biopsy. In comparison, only 1 of 36 Kveim tests in healthy subjects showed a papule at the test site. Secondly, among those with a *negative* microscopic reading, the mean papule size was greater in patients with active or inactive sarcoidosis than in patients with tuberculosis or healthy subjects. This suggests that the papule size provides information supplementary to the microscopic reading in the interpretation of the Kveim test result.

Mitchell *et al* (1969, 1970, 1974) and Mikhail and Mitchell (1971), using lot 5 of spleen K12, a suspension which in this validation study yielded the expected proportion of positive reactions in patients at different stages of sarcoidosis and a negligible proportion in those with active or quiescent pulmonary parenchymal tuberculosis or among healthy subjects, found positive reactions in an appreciable proportion of patients with certain other diseases, including Crohn's disease (Mitchell *et al*, 1969, 1970), ulcerative colitis (Mitchell *et al*, 1974), and tuberculous lymphadenitis (Mikhail and Mitchell,

1971). Moreover, they encountered a closely similar rate of positive reactions among patients with Crohn's disease after tests with an early batch (0025) of CSL suspension and with another lot (lot 14) derived from spleen K12 (Mitchell *et al*, 1970). A close concordance of results was also obtained with lot 5 (K12) and with batch 0042 (CSL) in tests among patients with ulcerative colitis (Mitchell *et al*, 1974), but at a lower rate of reactivity than in patients with Crohn's disease.

Hurley and Bartholomeusz (1968) reported their findings following an evaluation of an early batch of CSL suspension in which biopsy of the Kveim test site was performed if any visible or palpable nodule was detected. If no nodule was visible or palpable the result was read as negative. Positive reactions were encountered in 70% of 84 patients with histologically proven or clinically definite sarcoidosis and in less than 2% of 135 patients not suffering from sarcoidosis. Izumi *et al* (1974) reported their findings following the use of *early* 002 and 003 (0024-0031) and *late* 004 (004-2-004-5) batches of CSL Kveim suspension. Of the patients with pulmonary sarcoidosis who were Kveim tested within one year of presentation, 78% tested with batches 002 and 003 and 88% tested with batch 004 gave positive reactions. Among patients in whom the chest radiograph showed complete regression of sarcoidosis the difference in Kveim reactivity between the early and late batches was substantial: thus, of those tested with batches 002 and 003, only 27% were positive whereas 71% of those tested with the late batches 004 showed positive reactions. In patients with pulmonary diseases other than sarcoidosis (pulmonary tuberculosis, chronic obstructive lung diseases, silicosis, carcinoma, etc) no positive Kveim tests were encountered in 25 patients tested with the early batches (002 and 003), but of the 153 patients tested with later batches (004) 71 (53%) were positive.

More recently, Hurley *et al* (1975) have reported the results of a study in which biopsy of all Kveim test sites was obligatory and which included patients with a wide variety of diseases other than sarcoidosis: in this study batch 0017-0023, used in their original validation (Hurley and Bartholomeusz, 1968), and later batches 005-006, which had earlier been shown to give microscopically positive tests among patients with diseases other than sarcoidosis (Izumi *et al*, 1974), yielded positive tests among patients with sarcoidosis, tuberculosis, Hodgkin's disease, non-Hodgkin's lymphoma, Crohn's disease, ulcerative colitis, rheumatoid arthritis, and Weber-Christian disease. It is highly relevant that the *early* batch 0017-0023 used in the original validation produced the lowest percentage of positive reactions among

patients with diseases other than sarcoidosis and that these early batches showed only 12 (1.7%) microscopically positive responses among 722 patient with a wide variety of diseases other than sarcoidosis and Crohn's disease in the continued international Kveim test study (1966-1969) (Hurley and Bartholomeusz, 1971).

The fulfilment of the elaborate design of this present study, which required careful matching of subjects in different clinical categories, turned out to be extremely laborious and time-consuming. The advantages it brought were very slight—marginally more precise assessments of the relative reactivity of the Kveim test in active and inactive sarcoidosis and of its specificity. It contributed nothing to the assessment of any differences in reactivity between the four test suspensions on which the findings of this study are inconclusive. In retrospect it was a mistake to have adopted so demanding a design, and this should also have been clear in prospect.

Differences in reactivity between two test suspensions can be satisfactorily established only by testing substantial numbers of patients with sarcoidosis with the two suspensions in parallel. For this purpose active sarcoidosis cases are to be preferred to inactive cases because of their greater reactivity to Kveim test suspensions, as confirmed by the present study. This approach has been adopted in our subsequent investigations of these and other Kveim test suspensions. With regard to the selectivity of a suspension for sarcoidosis (Mitchell and Scadding, 1974), the difficulties in determining the criteria for an acceptable test suspension become increasingly apparent as additional Kveim tests are made with one particular lot and with sequential lots of test material from a 'validated' tissue source. We conclude that the possibility that positive reactions can be produced selectively in some other diseases following tests with a suspension that gives the expected proportions of reactions in patients with different stages of sarcoidosis and a negligible proportion of these in those with active or quiescent pulmonary tuberculosis must be considered.

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## Appendix

### MICROSCOPIC ASSESSMENT OF KVEIM TEST BIOPSIES

#### 1 Positive

The essential feature was the presence of one or more granulomas composed principally of epithelioid cells with occasional Langhans type giant cells. The overall appearances closely resembled those seen in sections from spontaneous sarcoid lesions in man.

#### 2 Equivocal

- (a) A diffuse arrangement of epithelioid cells with no true epithelioid cell granulomas.
- (b) Focal collections of histiocytes (with less abundant cytoplasm and smaller round nuclei) with few or no epithelioid cells.

#### 3 Negative

- (a) Non-specific inflammatory cells, mononuclear cells, lymphocytes, neutrophils, plasma cells, eosinophils
- (b) Foreign body reaction
- (c) Scar with fibroblasts or fibrocytes
- (d) Normal tissue